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L1: Entry 4 of 5 File: USPT Sep 25, 2001

US-PAT-NO: 6294330
DOCUMENT-IDENTIFIER: US 6294330 B1

TITLE: Protein fragment complementation assays for the detection of biological or drug interactions

DATE-ISSUED: September 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Michnick; Stephen William Watson	Westmount			CA
Remy; Ingrid	Montreal			CA

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Odyssey Pharmaceuticals Inc.	San Ramon	CA			02

APPL-NO: 09/ 124850 [PALM]
DATE FILED: July 30, 1998

PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 09/017,412 filed Feb. 2, 1998.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
CA	2196496	January 31, 1997

INT-CL: [07] C12 Q 1/68, C12 N 5/10, C12 N 1/21, C12 N 15/11, C12 N 15/63

US-CL-ISSUED: 435/6; 435/69.7, 435/325, 435/252.3, 435/254.11, 435/440, 435/455, 435/468, 435/320.1, 536/23.4, 536/23.5
US-CL-CURRENT: 435/6; 435/252.3, 435/254.11, 435/320.1, 435/325, 435/440, 435/455, 435/468, 435/69.7, 536/23.4, 536/23.5

FIELD-OF-SEARCH: 435/6, 435/69.7, 435/320.1, 435/325, 435/252.3, 435/254.11, 435/440, 435/455, 435/468, 536/23.4, 536/23.5

ART-UNIT: 166

PRIMARY-EXAMINER: Brusca; John S.

ATTY-AGENT-FIRM: Angres; Isaac

ABSTRACT:

The invention provides a general protein-fragment complementation assays to detect biomolecular interactions in vivo and in vitro. The protein-complemetation assay/universal reporter system can be used to detect and screen an agonist and an antagonist of a membrane receptor system. The assay can be used to study protein-protein, protein-DNA, protein-RNA, protein-carbohydrate, and protein-small molecule interactions. The assay can be used to screen cDNA libraries for binding of a target target protein with unknown proteins or libraries of small organic molecules for biological activity.

64 Claims, 12 Drawing figures

First Hit Fwd Refs

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L1: Entry 4 of 5 File: USPT Sep 25, 2001

US-PAT-NO: 6294330
DOCUMENT-IDENTIFIER: US 6294330 B1

TITLE: Protein fragment complementation assays for the detection of biological or drug interactions

DATE-ISSUED: September 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Michnick; Stephen William Watson	Westmount			CA
Remy; Ingrid	Montreal			CA

US-CL-CURRENT: 435/6; 435/252.3, 435/254.11, 435/320.1, 435/325, 435/440, 435/455, 435/468, 435/69.7, 536/23.4, 536/23.5

CLAIMS:

What is claimed is:

1. A method employing a Protein Complementation assay/Universal Reporter System (PCA/URS) for detecting and screening for ligands of a cellular receptor, which method comprises:

a) generating a first nucleic acid vector encoding a first fusion product comprising:

i) a first fragment of a first PCA/URS reporter molecule, and

ii) a second molecule, fused to said first fragment, which comprises a first subdomain of a cellular receptor molecule of interest;

b) generating a second nucleic acid vector encoding a second fusion product comprising:

i) a second fragment of said first PCA/URS reporter molecule, and

ii) a third molecule, fused to said second fragment, which comprises a second subdomain of said cellular receptor, and where said second subdomain may be the same as said first subdomain in the case of a homodimeric cellular receptor, or different from said first subdomain in the case of a heterodimeric cellular receptor; or a receptor coactivator or a protein;

c) transfecting prokaryotic or eukaryotic cells with said first and second nucleic acid vectors; and

d) testing said transfected cells for activity of said PCA/URS reporter molecule, said activity indicating reassociation of the first and second fragments of the PCA/URS reporter molecule

mediated by the interaction of said first and second subdomains of the cellular receptor molecule; said association being induced by binding said receptor to said ligand.

2. A method employing a Protein Complementation Assay/Universal Reporter System (PCA/URS) for detecting and screening for ligands of a cellular receptor, which method comprises:

a) generating a first nucleic acid vector encoding a first fusion product comprising:

i) a first fragment of a first PCA/URS reporter molecule, and

ii) a second molecule, fused to said first fragment, which comprises a first subdomain of a cellular receptor molecule of interest;

b) generating a second nucleic acid vector encoding a second fusion product comprising:

i) a second fragment of said first PCA/URS reporter molecule, and

ii) a third molecule, fused to said second fragment, which comprises a second subdomain of said cellular receptor, and where said second subdomain may be the same as said first subdomain in the case of a homodimeric cellular receptor, or different from said first subdomain in the case of a heterodimeric cellular receptor;

c) transfecting prokaryotic or eukaryotic cells with said first and second nucleic acid vectors;

d) obtaining a clonal population of cells that express said first and second fusion products; and

e) testing said transfected cells for activity of said PCA/URS reporter molecule, said activity indicating reassociation of the first and second fragments of the PCA/URS reporter molecule mediated by the interaction of said first and second subdomains of the cellular receptor molecule; said association being induced by binding said receptor to said ligand.

3. The method of claim 2, further comprising the step of treating said clonal population of cells with a chemical composition prior to said testing of the cells for PCA/URS activity, thus measuring the ability of the chemical composition to induce or inhibit the activity.

4. The method of claim 3, wherein said chemical composition is an individual compound or a mixture of compounds obtained from a chemical compound library or combinatorial chemical synthesis.

5. The method of claim 2, wherein said reporter molecule is a multimeric protein.

6. The method of claim 2, wherein said reporter molecule is a multimeric receptor.

7. The method of claim 2, wherein said reporter molecule is a multimeric bindng protein.

8. The method of claim 2, wherein said reporter molecule is a catalytic molecule.

9. The method of claim 2, wherein said reporter molecule is an energy transfer molecule.

10. The method of claim 2, wherein said reporter molecule is a fluorescent, luminescent or phosphorescent protein.

11. The method of claim 2, wherein said reporter molecule is an electron transfer molecule.

12. The method of claim 2, wherein said reporter molecule is a chemiluminescent molecule.

13. The method of claim 3, wherein said chemical composition is a ligand agonist or antagonist.

14. The method of claim 3, wherein said chemical composition is a nucleic acid.

15. The method of claim 3, wherein said chemical composition is a peptide.

16. The method of claim 3, wherein said chemical composition is a carbohydrate.

17. The method of claim 3, wherein said chemical composition is a natural product or extract.

18. The method of claim 4, wherein said library of compounds is a combinatorial nucleic acid library.

19. The method of claim 4, wherein said library of compounds is a combinatorial carbohydrate library.

20. The method of claim 4, wherein said library of compounds is a combinatorial peptide or protein library.

21. The method of claim 3, wherein in the treatment step the cells are treated with the chemical composition at different concentrations in the medium, and the PCA/URS activity is compared at the different concentrations.

22. The method of claim 21, wherein the values of PCA/URS activity versus concentration of the chemical composition are used to estimate the binding isotherm of the composition to the cellular receptor.

23. The method of claim 2, wherein the PCA/URS activity is detected using a fluorescent assay, and the activity is monitored by fluorescence microscopy, fluorescent cell sorting (FACS), or by spectroscopy of aliquots of the cells.

24. The method of claim 21, wherein said reporter molecule is dihydrofolate reductase and said detection method comprises treatment of the cells with fluorescein-conjugated methotrexate before monitoring the cellular fluorescence.

25. The method of claim 2, wherein said cellular receptor is the Erythropoietin receptor.

26. The method of claim 2, wherein said cellular receptor is a naturally occuring protein which upon binding a ligand induces a cellular response.

27. The method of claim 2, wherein said cellular receptor is an enzyme which is activated by

binding a ligand.

28. The method of claim 2, wherein said cellular receptor is a natural or synthetic protein which undergoes conformational change or oligomerizes upon binding a ligand.

29. The method of claim 2, wherein said cellular receptor is a member of the cytokine receptor superfamily.

30. The method of claim 2, wherein said cellular receptor is the receptor for an interleukin or cytokine.

31. The method of claim 2, wherein said cellular receptor is a hormone receptor.

32. The method of claim 2, wherein said cellular receptor is a receptor for a member of a protein family selected from the group consisting of the TGF-beta, NGF, FGF/HBGF, chemokine, IL-6, LIF/OSM, TNF, and MDK/PTN families.

33. The method of claim 2, wherein said cellular receptor is the receptor for a member of the tumor growth factor beta family.

34. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of the forms of TGF-beta, Mullerian inhibitory substance (MIS), the inhibins (INHA and INHB), the bone morphogenic proteins (BMP), the growth development factors (GDF-1, GDF-3, GDF-5, GDF-6, GDF-7 and GDF-8), endometrial bleeding associated factor (EBAF/Lefty), and glial cell line-derived neurotrophic factor (GDNF).

35. The method of claim 2, wherein said cellular receptor is the receptor for a member of the nerve growth factor family.

36. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4, and NT-5.

37. The method of claim 2, wherein said cellular receptor is the receptor for a member of the fibroblast growth factor and heparin-binding growth factor family.

38. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of fibroblast growth factor-3 (FGF-3), FGF-4 (int-2), FGF-5, FGF-6 (hst-2), keratinocyte growth factor (KGF/FGF-7), androgen-induced growth factor (AIGF/FGF-8), glia-activating factor (GAF/FGF-9), FGF-11, FGF-12, FGF-13, and FGF-14.

39. The method of claim 2, wherein said cellular receptor is the receptor for a member of the chemokine family.

40. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of platelet factor 4 (PF4), platelet basic protein (PBP), monocyte-derived neutorphil chemotactic factor (MDNCF/IL-8), melanoma growth stimulatory activity protein (MGSA), macrophage inflammatory protein 2 (MIP-2), Mig, chicken 9E3, pig aveolar macrophage chemotactic factor, pre-B cell growth stimulatory factor (PBSF), cytokine-induced neutrophil chemoattractant-2, and IP10.

41. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of monocyte chemotactic protein 1, (MCP-1), MCP-2, MCP-3, MCP-4, MCP-5, MIP-1-alpha, MIP-1-beta, MIP-1-gamma, MIP-3-alpha, MIP-3-beta, MIP-4, MIP-5, RANTES, SIS-epsilon, thymus and activation-regulated chemokine (TARC), eotaxin, I-309, HCC-1/NCC-2, HCC-3, 6Ckine/Exodus-2/SLC, thymus-expressed chemokine (TECK) and mouse protein C10.

42. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of fractalkine and GCP-2/LIX.

43. The method of claim 2, wherein said cellular receptor is a member of the group consisting of CXCR-1, CXCR-2, CXCR-3, CXCR-4, CCR-1, CCR-2, CCR-3, CCR-4, CCR-5, CCR-6, CCR-7, CCR-8, and CX3CR.

44. The method of claim 2, wherein said cellular receptor is the receptor for a member of the interleukin-6 (IL-6) family.

45. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of IL-6, granulocyte colony-stimulating factor (G-CSF), and myelomonocytic growth factor (MGF).

46. The method of claim 2, wherein said cellular receptor is the receptor for a member of the leukemia inhibitory factor and oncostatin family.

47. The method of claim 2, wherein said cellular receptor is the receptor for a member of the group selected from leukemia inhibitory factor (LIF) and oncostatin (OSM).

48. The method of claim 2, wherein said cellular receptor is the receptor for a member of the tumor necrosis factor family.

49. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of tumor necrosis factor alpha (TNF-a), tumor necrosis factor beta (TNF-b/LT-a), CD40L, CD137L/4-1BBL, CD134L/OX40L, CD27L/CD70, FasL, CD30L, LT-b, and TNF-related apoptosis-inducing ligand (TRAIL).

50. The method of claim 2, wherein said cellular receptor is a receptor selected from the group consisting of LNGFR/p75, CD40, CD137/4-1BB/ILA, TNFRI/p55/CD120a, TNFRII/p75/CD120b, CD134/OX40/ACT35, CD27, Fas/CD95/APO-1, CD30/Ki-1, LT-betaR, DR3/WSL-1/TRAMP/APO-3/LARD, DR4, DR5, DcR1/TRID, TR2, GITR, and osteoprotegerin (OPG).

51. The method of claim 2, wherein said cellular receptor is the receptor for a member of the midkine and pleiotrophin family.

52. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of midkine (MK), pleiotrophin (PTN), chicken retinoic acid-induced heparin-binding protein (RI-MB), and Xenopus pleiotrophic factors alpha-1, alpha-2, beta-1, and beta-2.

53. The method of claim 2, wherein said cellular receptor is a member of the family of G-protein-coupled receptors.

54. The method of claim 2, wherein said cellular receptor is a receptor for transferrin.

55. The method of claim 2, wherein said cellular receptor is a receptor for a member of the group consisting of macrophage stimulating protein, hepatocyte growth factor, platelet-derived growth factor, insulin-like growth factor, and platelet-derived endothelial cell growth factor.

56. The method of claim 2, wherein said cellular receptor is the receptor for a steroid hormone.

57. The method of claim 2, wherein said cellular receptor is the receptor for an eicosanoid hormone.

58. The method of claim 2, wherein said cellular receptor has been identified from an expressed sequence tag (EST) nucleic acid sequence.

59. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of IL-1 a, IL-1 b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, and IL-18.

60. The method of claim 2 wherein said receptor is a nuclear receptor or coativator of said nuclear receptor.

61. The method of claim 60 wherein said receptor is a Vitamin D receptor.

62. The method of claim 60 wherein said receptor is a Vitamin A or a retinoid associated receptor.

63. The method of claim 60 wherein said receptor is a Gamma PPAR.

64. The method of claim 60 wherein said receptor is a steroid receptors.

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L1: Entry 4 of 5 File: USPT Sep 25, 2001

DOCUMENT-IDENTIFIER: US 6294330 B1
TITLE: Protein fragment complementation assays for the detection of biological or drug interactions

CLAIMS:

34. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of the forms of TGF-beta, Mullerian inhibitory substance (MIS), the inhibins (INHA and INHB), the bone morphogenic proteins (BMP), the growth development factors (GDF-1, GDF-3, GDF-5, GDF-6, GDF-7 and GDF-8), endometrial bleeding associated factor (EBAF/Lefty), and glial cell line-derived neurotrophic factor (GDNF).

First Hit Fwd Refs

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L1: Entry 2 of 5 File: USPT Oct 29, 2002

US-PAT-NO: 6472179
DOCUMENT-IDENTIFIER: US 6472179 B2

TITLE: Receptor based antagonists and methods of making and using

DATE-ISSUED: October 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stahl; Neil	Carmel	NY		
Yancopoulos; George D.	Yorktown Heights	NY		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Regeneron Pharmaceuticals, Inc.	Tarrytown	NY			02

APPL-NO: 09/ 313942 [PALM]
DATE FILED: May 19, 1999

PARENT-CASE:
This application claims priority of U.S. Provisional application No. 60/101,858 filed Sep. 25, 1998. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

INT-CL: [07] C12 P 21/04, C07 H 21/04

US-CL-ISSUED: 435/69.7; 435/320.1, 435/252.3, 435/254.2, 435/325, 435/348, 435/365, 435/361, 435/328, 435/335, 536/23.5 , 536/23.4, 530/350, 530/388.22, 514/2, 424/179.1
US-CL-CURRENT: 435/69.7; 424/179.1, 435/252.3, 435/254.2, 435/320.1, 435/325, 435/328, 435/335, 435/348, 435/361, 435/365, 514/2, 530/350, 530/388.22, 536/23.4, 536/23.5

FIELD-OF-SEARCH: 424/179.1, 530/350, 530/388.22, 514/2, 536/23.5, 536/23.4, 435/335, 435/320.1, 435/325, 435/252.3, 435/348, 435/361, 435/365, 435/254.2, 435/328, 435/69.7

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5262522	November 1993	Gearing	530/350
☐	5426048	June 1995	Gearing	435/252
☐	5470952	November 1995	Stahl et al.	530/350
☐	5510259	April 1996	Sugamura et al.	435/240.2
☐	5599905	February 1997	Mosley et al.	530/350
☐	5844099	December 1998	Stahl et al.	530/350
☐	6143871	November 2000	Bonnefoy et al.	530/351

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0367566	May 1997	EP	
WO 93/10151	May 1993	WO	
WO 96/11213	April 1996	WO	

OTHER PUBLICATIONS

The Leukocyte Antigen FactsBook, Barclay et al., editors. Academic Press, Harcourt Brace Jovanovich, Publishers, 1993, pp. 162, 166, 188, 290, 320, 322, 330, 338 and 410.*
Sato, et al., Current Opinions in Cell Biology, 1994, 6: 174-179.
Miyajima, et al., Annual Review of Immunology, 1992, 10: 295-331.
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Bassing, et al., Journal of Biological Chemistry, 1994, 269: 14861-14864.
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Kennedy and Park, Journal of Clinical Immunology, 1996, 16: 134-143.
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Immunobiology, The Immune System in Health and Disease, 2nd Edition, by Charles A. Janeway, Jr. And Paul Travers, published by Current Biology Lt./ Garland Publishing In., copyright 1996.

ART-UNIT: 1646

PRIMARY-EXAMINER: Spector; Lorraine

ASSISTANT-EXAMINER: O'Hara; Eileen B.

ATTY-AGENT-FIRM: Cobert; Robert J. Kempler; Gail M. Palladino; Linda O.

ABSTRACT:

The present invention provides a fusion polypeptide capable of binding a cytokine to to form a nonfunctional complex. It also provides a nucleic acid sequence encoding the fusion polypeptide and methods of making and uses for the fusion polypeptide.

25 Claims, 71 Drawing figures

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L1: Entry 2 of 5 File: USPT Oct 29, 2002

US-PAT-NO: 6472179
DOCUMENT-IDENTIFIER: US 6472179 B2

TITLE: Receptor based antagonists and methods of making and using

DATE-ISSUED: October 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stahl; Neil	Carmel	NY		
Yancopoulos; George D.	Yorktown Heights	NY		

US-CL-CURRENT: 435/69.7; 424/179.1, 435/252.3, 435/254.2, 435/320.1, 435/325, 435/328, 435/335, 435/348, 435/361, 435/365, 514/2, 530/350, 530/388.22, 536/23.4, 536/23.5

CLAIMS:

We claim:

1. An isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising: a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of a receptor for the cytokine; b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of a receptor for the cytokine; and c) a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component, wherein the receptor of a) can be the same or different from the receptor of b).

2. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component.

3. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component.

4. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, and leukemia inhibitory factor.

5. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the interferon family of cytokines selected from the group consisting of IFN-gamma,

IFN-alpha, and IFN-beta.

6. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the immunoglobulin superfamily of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

7. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.

8. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TGF-.beta./BMP family selected from the group consisting of TGF-.beta.1, TGF-.beta.2, TGF-.beta.3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.

9. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14 and MIF.

10. The isolated nucleic acid molecule of claim 1, wherein the multimerizing component comprises an immunoglobulin derived domain.

11. The isolated nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

12. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 1.

13. A composition capable of binding a cytokine to form a nonfunctional complex comprising a multimer of the fusion polypeptide of claim 12.

14. The composition of claim 13, wherein the multimer is a dimer.

15. A vector which comprises the nucleic acid molecule of claim 1.

16. An expression vector comprising a nucleic acid molecule of claim 1, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.

18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell.

19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.

20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.

21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.

22. The host-vector system of claim 17, wherein the suitable host cell is a 293 cell.

23. The host-vector system of claim 17, wherein the suitable host cell is a BHK cell.

24. The host-vector system of claim 17, wherein the suitable host cell is a NS0 cell.

25. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 17, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

Hit List

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Search Results - Record(s) 1 through 5 of 5 returned.

☐ 1. Document ID: US 6649588 B1

L1: Entry 1 of 5 File: USPT Nov 18, 2003

DOCUMENT-IDENTIFIER: US 6649588 B1
TITLE: Inhibition of TGF-.beta. and uses thereof

CLAIMS:

1. A method for inhibiting activity of TGF-.beta., comprising bringing tissue expressing TGP-.beta. in direct contact with an amount of an ebaf protein effective to inhibit the activity of TGF-.beta., wherein the ebaf protein has the amino acid sequence set forth in SEQ ID NO:2.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

☐ 2. Document ID: US 6472179 B2

L1: Entry 2 of 5 File: USPT Oct 29, 2002

DOCUMENT-IDENTIFIER: US 6472179 B2
TITLE: Receptor based antagonists and methods of making and using

CLAIMS:

8. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TGF-.beta./BMP family selected from the group consisting of TGF-.beta.1, TGF-.beta.2, TGF-.beta.3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De

☐ 3. Document ID: US 6294662 B1

L1: Entry 3 of 5 File: USPT Sep 25, 2001

DOCUMENT-IDENTIFIER: US 6294662 B1
TITLE: Nucleic acids encoding an endometrial bleeding associated factor (ebaf)

CLAIMS:

1. An isolated nucleic acid molecule encoding an endometrial bleeding associated factor (ebaf) having the nucleic acid sequence shown in SEQ ID NO. 1.

5. A 1.5 kb mRNA transcript encoding an isoform of ebaf.

6. A 2.5 kb mRNA transcript encoding an isoform of ebaf.

7. An isolated nucleic acid molecule of claim 1 which is a probe for detecting the presence of ebaf nucleic acid in bodily samples.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De

☐ 4. Document ID: US 6294330 B1

L1: Entry 4 of 5 File: USPT Sep 25, 2001

DOCUMENT-IDENTIFIER: US 6294330 B1
TITLE: Protein fragment complementation assays for the detection of biological or drug interactions

CLAIMS:

34. The method of claim 2, wherein said cellular receptor is the receptor for a protein selected from the group consisting of the forms of TGF-beta, Mullerian inhibitory substance (MIS), the inhibins (INHA and INHB), the bone morphogenic proteins (BMP), the growth development factors (GDF-1, GDF-3, GDF-5, GDF-6, GDF-7 and GDF-8), endometrial bleeding associated factor (EBAF/Lefty), and glial cell line-derived neurotrophic factor (GDNF).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De

☐ 5. Document ID: US 5916751 A

L1: Entry 5 of 5 File: USPT Jun 29, 1999

DOCUMENT-IDENTIFIER: US 5916751 A
TITLE: Method for the diagnosis of selected adenocarcinomas

CLAIMS:

1. A method for diagnosing a serous or mucinous adenocarcinoma in a human comprising comprising the steps of:

removing a bodily sample from the human; and

assaying the bodily sample for elevated expression above normal of a gene designated designated endometrial bleeding associated factor (ebaf).

3. A method for diagnosing a serous or mucinous adenocarcinoma in a human female not suffering from abnormal uterine bleeding, comprising the steps of:

removing a bodily sample from the human female after the completion of the human female's menstrual period; and

assaying the bodily sample for elevated expression above normal of a gene designated endometrial bleeding associated factor (ebaf).

9. A method for diagnosing a serous or mucinous adenocarcinoma in a human male comprising the steps of:

removing a bodily sample from the human; and

assaying the bodily sample for elevated expression above normal of a gene designated endometrial bleeding associated factor (ebaf).

Terms	Documents
ebaf.clm.	5

First Hit Fwd Refs

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L1: Entry 5 of 5 File: USPT Jun 29, 1999

US-PAT-NO: 5916751
DOCUMENT-IDENTIFIER: US 5916751 A

TITLE: Method for the diagnosis of selected adenocarcinomas

DATE-ISSUED: June 29, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tabibzadeh; Siamak	Tampa	FL		
Kothapalli; Ravi	Tampa	FL		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
University of South Florida	Tampa	FL			02

APPL-NO: 08/ 919421 [PALM]
DATE FILED: August 27, 1997

PARENT-CASE:
CROSS REFERENCE This application claims the benefit of U.S. Provisional Application No. 60/025,800, entitled Method for the Early diagnosis of Colon Cancer, Abnormal Uterine Bleeding, or Pregnancy filed Aug. 27, 1996.

INT-CL: [06] G01 N 33/574, G01 N 33/48, C12 Q 1/68

US-CL-ISSUED: 435/6; 435/7.23, 436/64, 436/813
US-CL-CURRENT: 435/6; 435/7.23, 436/64, 436/813

FIELD-OF-SEARCH: 435/6, 435/7.23, 436/64, 436/813

PRIOR-ART-DISCLOSED:

OTHER PUBLICATIONS

RNA Image Kit Product Brochure, 1993, pp. 1-21.
"DNA Sequencing by the Dideoxy Method," Sequences Ver. 2.0 A New Genetically Engineered Enzyme for DNA Sequencing, U.S. Biochemical Corp., 1988-89, pp. 7-29-7-35.
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Analysis of RNA by Northern and Slot Blot Hybridization, Current Protocols in Molecular Biology, Unit 4.9, 1993, pp. 4.9.1-4.9.14.
Ravi Kothapalli, Ibrahim Buyuksal, Shi-Qu Wu, Nasser Chegini and Siamak Tabibzadeh,

"Detection of ebaf, a Novel Human Gene of the Transforming Growth Factor B Superfamily," Rapid Publication, The American Society for Clinical Investigation, Inc., vol. 99, No. 10, May 1997, pp. 2342-2350.
Siamak Tabibzadeh, Ravi Kothapalli, Ibrahim Buyuksal, "Distinct Tumor Specific Expression of TGFB4 (ebaf), a Novel Human Gene of the TGF-B Superfamily," Frontiers in Bioscience 2, Jul. 1997, pp. 18-25.
Amersham Life Science Brochure, 1994, pp. 1-29.

ART-UNIT: 162

PRIMARY-EXAMINER: Scheiner; Toni R.

ASSISTANT-EXAMINER: Bansal; Geetha P.

ATTY-AGENT-FIRM: Kohn & Associates

ABSTRACT:

A method for the early diagnosing of selected adenocarcinomas in a human comprising the steps of removing a bodily sample from the human, and assaying the bodily sample sample for elevated expression of a specific gene, the gene being assayed for in the the bodily sample is the TGFB-4 gene (hereinafter referred to as the endometrial bleeding associated factor (ebaf) gene. The bodily sample can be tissue from a specific organ in the body, or a blood sample. Increased levels of ebaf in the sample relative to basal levels may be indicative of a mucinous adenocarcinoma of the colon or ovaries, or an adenocarcinoma of the testis.

14 Claims, 3 Drawing figures

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Search Results - Record(s) 1 through 10 of 10 returned.

☐ 1. WO009955902A1. 29 Apr 99. 04 Nov 99. DIAGNOSTIC MARKERS OF HUMAN FEMALE INFERTILITY. TABIBZADEH, SIAMAK. C12Q001/00;.

☐ 2. WO 200244732A. Diagnosing presence of or predisposition for allergic disease such as allergic asthma, atopic dermatitis, utilizes expression profiling images of activated lymphocytes or monocytes/macrophages. BLASER, K, et al. C12Q001/68 G01N033/50 G01N033/569.

☐ 3. WO 200234281A. Promoting hair growth in a subject, useful for preventing or treating hair loss and all types of alopecia, comprises administering endometrial bleeding associated factor or its analog, or modulator of their expression. MASON, J M, et al. A61K038/00 C07H021/04.

☐ 4. WO 200229105A. Inhibiting the activity of transforming growth factor (TGF) beta, for treating e.g. fibrosis, comprises contacting tissue expressing TGF beta with ebaf peptide its analogue. MASON, J M, et al. A01N025/00 A01N037/18 A61K038/00 C07K001/00 C07K014/00 C07K017/00 C12Q001/68 G01N033/574.

☐ 5. US 6294662B. New nucleic acid molecule encoding endometrial bleeding associated factor, useful in early diagnosis of selected adenocarcinomas in human, e.g. adenocarcinomas of colon, ovaries or testis. TABIBZADEH, S. C07H021/04 C12Q001/68.

☐ 6. WO 200101134A. New isolated nucleic acid useful for diagnosing colon, testicular, and ovarian cancer. TABIBZADEH, S. G01N033/53.

☐ 7. WO 200066068A. Inducing growth and enhancing survival of nervous tissue by contacting with endometrial bleeding associated factor protein. TABIBZADEH, S. A61K000/00 A61K031/05 A61K031/203 A61K031/57 A61K035/76 A61K038/00 A61K045/00 A61K048/00 A61P025/14 A61P025/16 A61P025/28 C07J009/00 C12N005/10 C12N015/09.

☐ 8. WO 9955902A. Diagnosis of endometrial irregularities by detecting ebaf or its splice variants, particularly for diagnosing infertility. TABIBZADEH, S. C12Q001/00.

☐ 9. US 5916751A. Detecting serous or mucinous colon/ovarian adenocarcinomas and testicular adenocarcinoma by assaying for elevated expression of a gene. KOTHAPALLI, R, et al. C12Q001/68 G01N033/48 G01N033/574.

☐ 10. WO 9914327A. Antibodies against specific proteins overexpressed in tumors. CHEN, J, et al. A61K000/00 A61K038/00 A61K039/395 A61K045/00 A61P001/00 A61P013/12 A61P017/00 A61P017/06 A61P025/00 A61P025/16 A61P025/28 A61P031/12 A61P035/00 A61P043/00 C07K014/435 C07K014/47 C07K014/475 C07K014/50 C07K014/705 C07K016/18 C07K016/22 C07K016/28 C07K019/00 C12N001/19 C12N001/21 C12N005/10 C12N015/02 C12N015/09 C12N015/12 C12N015/18 C12N015/52 C12N015/62 C12N015/63 C12P021/00 C12P021/02 C12P021/08 C12Q001/68 G01N033/53 C12P021/02 C12P021/02 C12P021/02 C12R001:19 C12R001:645 C12R001:91 C12N001/19 C12N001/21 C12N005/10 C12P021/02 C12P021/02 C12P021/02 C12P021/08 C12R001:19 C12R001:19 C12R001:645 C12R001:645 C12R001:91 C12R001:91 C12R001:91 C12P021/02 C12P021/02 C12P021/02 C12R001:19 C12R001:645

C12R001:91 C12N001/19 C12N001/21 C12N005/10 C12R001:19 C12R001:645 C12R001:91 C12N001/19 C12N001/21 C12N005/10 C12R001:19 C12R001:645 C12R001:91.

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Terms	Documents
L2 not L1	10

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L3: Entry 1 of 10 File: EPAB Nov 4, 1999

PUB-NO: WO0009955902A1
DOCUMENT-IDENTIFIER: WO 9955902 A1
TITLE: DIAGNOSTIC MARKERS OF HUMAN FEMALE INFERTILITY

PUBN-DATE: November 4, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
TABIBZADEH, SIAMAK	US

ASSIGNEE-INFORMATION:

NAME	COUNTRY
UNIV SOUTH FLORIDA	US
TABIBZADEH SIAMAK	US

APPL-NO: US09909366
APPL-DATE: April 29, 1999

PRIORITY-DATA: US08341898P (April 29, 1998)

INT-CL (IPC): C12 Q 1/00
EUR-CL (EPC): G01N033/68; C07K014/47, C12Q001/68

ABSTRACT:

CHG DATE=19991202 STATUS=O>There is provided a method for diagnosing endometrial irregularites particularly infertility by screening an endometrial sample or bodily fluid for the presence of ebaf. Also provided is a diagnostic tool for determining the presence of endometrial irregularities screens a sample for the presence of ebaf. A contraceptive containing an effective amount of ebaf and a pharmaceutically acceptable carrier is also provided. Additionally, a diagnostic kit for timing conception is provided having a screening tool for screening a sample for the present of ebaf. Also provided is a method of treating endometrial irregularities by by down-regulating the expression of ebaf.

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L1: Entry 1 of 5 File: USPT Nov 18, 2003

US-PAT-NO: 6649588
DOCUMENT-IDENTIFIER: US 6649588 B1

TITLE: Inhibition of TGF-.beta. and uses thereof

DATE-ISSUED: November 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tabibzadeh; Siamak	Albertson	NY		
Mason; James M.	Bethpage	NY		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
North Shore - Long Island Jewish Research Institute	Manhasset	NY			02

APPL-NO: 09/ 679971 [PALM]
DATE FILED: October 5, 2000

INT-CL: [07] A61 K 38/00, A01 N 25/00, C07 K 17/00

US-CL-ISSUED: 514/2; 514/21, 514/899, 530/350
US-CL-CURRENT: 514/2; 514/21, 514/899, 530/350

FIELD-OF-SEARCH: 514/2, 514/44, 514/21, 514/899, 530/350

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5821227	October 1998	Dennis et al.	
☐	5916751	June 1999	Tabibzadeh et al.	

OTHER PUBLICATIONS

Crystal, transfer of genes to humans: early lessons and obstacles to success, 1995, SCIENCE, vol. 270, pp. 404-409.*
Giorgio Palu et al. In pursuit of new developments for gene therapy of human diseases Journal of Biotechnolgy 68 1999 1-13.*

Tabibzadeh, S., et al., Dysregulated Expression of ebaf, a Novel Molecular Defect in in the Endometria of Patients with Infertility, The Journal of Clinical Endocrinology & Metabolism, 2000, vol. 85, No. 7, pp. 2526-2536.

ART-UNIT: 1636

PRIMARY-EXAMINER: Yucel; Remy

ASSISTANT-EXAMINER: Katcheves; Konstantina

ATTY-AGENT-FIRM: Amster, Rothstein & Ebenstein LLP

ABSTRACT:

The present invention provides a method for inhibiting activity of TGF-.beta., comprising contacting tissue expressing TGF-.beta. with an amount of ebaf or an ebaf ebaf analogue. The present invention further provides a method for treating a condition associated with overactivity of TGF-.beta., particularly fibrosis, a defect in cell proliferation, or a coagulation defect. The present invention also provides a method for inhibiting activity of TGF-.beta., comprising contacting tissue expressing TGF-.beta. with a modulator of ebaf expression, or a modulator of expression of an ebaf analogue. The present invention is further directed to a method for treating fibrosis in a subject in need of treatment, comprising administering to the subject an amount of ebaf or an ebaf analogue effective to treat the fibrosis. Finally, the present invention provides a method for treating a defect in cell proliferation in a subject in need of treatment, comprising administering to the subject an amount of ebaf or an ebaf analogue effective to treat the defect in cell proliferation.

17 Claims, 27 Drawing figures

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L1: Entry 1 of 5 File: USPT Nov 18, 2003

US-PAT-NO: 6649588
DOCUMENT-IDENTIFIER: US 6649588 B1

TITLE: Inhibition of TGF-.beta. and uses thereof

DATE-ISSUED: November 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tabibzadeh; Siamak	Albertson	NY		
Mason; James M.	Bethpage	NY		

US-CL-CURRENT: 514/2; 514/21, 514/899, 530/350

CLAIMS:

What is claimed is:

1. A method for inhibiting activity of TGF-.beta., comprising bringing tissue expressing TGP-.beta. in direct contact with an amount of an ebaf protein effective to inhibit the activity of TGF-.beta., wherein the ebaf protein has the amino acid sequence set forth in SEQ ID NO:2.

2. The method of claim 1, wherein the contacting is effected in vivo.

3. The method of claim 2, wherein the contacting is effected in vivo in a mammal.

4. The method of claim 3, wherein the mammal is a human.

5. The method of claim 4, wherein the human has a condition associated with overactivity of TGF-.beta..

6. The method of claim 5, wherein the condition is fibrosis.

7. The method of claim 6, wherein the fibrosis is a scar, a keloid, cirrhosis, Asherman's syndrome, Meigs' syndrome, a muscular dystrophy, an autoimmune disorder, post-surgical fibrosis, or primary pulmonary fibrosis.

8. The method of claim 7, wherein the scar results from a burn, radiation, a chemical, or a myocardial infarct.

9. The method of claim 7, wherein the muscular dystrophy is Duchenne muscular dystrophy.

10. The method of claim 7, wherein the autoimmune disorder is scleroderma.

11. The method of claim 7, wherein the primary pulmonary fibrosis is Hamman Rich Syndrome or retroperitoneal fibrosis.

12. The method of claim 5, wherein the condition is a defect in cell proliferation.

13. The method of claim 12, wherein the defect in cell proliferation is hyperplasia or neoplasia.

14. The method of claim 5, wherein the condition is a coagulation defect.

15. The method of claim 14, wherein the coagulation defect is menstrual bleeding, abnormal uterine bleeding, coagulopathy, or toxemia of pregnancy.

16. The method of claim 1, wherein the contacting is effected ex vivo.

17. The method of claim 3, wherein the mammal is immunocompromised.

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L1: Entry 3 of 5 File: USPT Sep 25, 2001

US-PAT-NO: 6294662
DOCUMENT-IDENTIFIER: US 6294662 B1

TITLE: Nucleic acids encoding an endometrial bleeding associated factor (ebaf)

DATE-ISSUED: September 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tabibzadeh; Siamak	Searingtown	NY		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
University of South Florida	Tampa	FL			02

APPL-NO: 09/ 342819 [PALM]
DATE FILED: June 29, 1999

PARENT-CASE:
CROSS REFERENCE This application is a continuation in part of Ser. No. 08/919,421, filed Aug. 27, 1997 now U.S. Pat. No. 5,916,751; which claims the benefit of provisional application Ser. No. 60/025,800, filed Aug. 27, 1996, incorporated herein by reference.

INT-CL: [07] C07 H 21/04, C12 Q 1/68

US-CL-ISSUED: 536/23.5; 536/23.1, 536/24.31, 536/24.33, 435/6
US-CL-CURRENT: 536/23.5; 435/6, 536/23.1, 536/24.31, 536/24.33

FIELD-OF-SEARCH: 435/6, 435/7.1, 435/320.1, 536/23.5, 536/24.31, 536/24.33, 536/23.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	6027917	February 2000	Celeste et al.	435/69.1

OTHER PUBLICATIONS

Kothapalli. Journal of Clinical Investigation. 99:2342-2350, May 1997.*

Tabibzadeh et al. Frontiers in Bioscience. 2:18-25, Jul. 1997.*
Hillier et al. GenBank Accession No. R37562, May 1995.*
Frigerio et al. GenBank Accession No. T25016, May 1997.

ART-UNIT: 165

PRIMARY-EXAMINER: Myers; Carla J.

ATTY-AGENT-FIRM: Kohn & Associates

ABSTRACT:

A method for the early diagnosing of selected adenocarcinomas in a human comprising the steps of removing a bodily sample from the human, and assaying the bodily sample sample for elevated expression of a specific gene. The gene being assayed for in the the bodily sample is the TGFB-4 gene (hereinafter referred to as the endometrial bleeding associated factor (ebaf) gene. The bodily sample can be tissue from a specific organ in the body, or a blood sample. Increased levels of ebaf in the sample relative to basal levels may be indicative of a mucinous adenocarcinoma of the colon or ovaries, or an adenocarcinoma of the testis.

7 Claims, 3 Drawing figures

First Hit Fwd Refs

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L1: Entry 3 of 5 File: USPT Sep 25, 2001

US-PAT-NO: 6294662
DOCUMENT-IDENTIFIER: US 6294662 B1

TITLE: Nucleic acids encoding an endometrial bleeding associated factor (ebaf)

DATE-ISSUED: September 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tabibzadeh; Siamak	Searingtown	NY		

US-CL-CURRENT: 536/23.5; 435/6, 536/23.1, 536/24.31, 536/24.33

CLAIMS:

What is claimed is:

1. An isolated nucleic acid molecule encoding an endometrial bleeding associated factor (ebaf) having the nucleic acid sequence shown in SEQ ID NO. 1.

2. An isolated nucleic acid molecule of claim 1 which is a DNA molecule.

3. An isolated nucleic acid molecule of claim 1 which is a cDNA molecule.

4. An isolated nucleic acid molecule of claim 1 which is an RNA molecule.

5. A 1.5 kb mRNA transcript encoding an isoform of ebaf.

6. A 2.5 kb mRNA transcript encoding an isoform of ebaf.

7. An isolated nucleic acid molecule of claim 1 which is a probe for detecting the presence of ebaf nucleic acid in bodily samples.